L3: Entry 17 of 25 File: USPT Jun 26, 2001

DOCUMENT-IDENTIFIER: US 6251614 B1
TITLE: Rapid methods for identifying modifiers of cellular apoptosis activity

Brief Summary Text (5):
In regard to the latter biochemical event, proteases within the cysteine aspartic acid protease family, also known as the ICE/CED-3 family, are critical for effecting the process of apoptosis. These enzymes are cysteine proteases and exhibit substrate specificity for cleavage after an aspartic acid residue. Due to these characteristics, these enzymes are now referred to by the above term "cysteine aspartic acid proteases" or "caspases". During the apoptotic process, caspase activity is generated in cells and known inhibitors of the caspase family of proteases inhibit apoptosis.

Detailed Description Text (4):
In another embodiment, the invention is directed to a method for identifying compounds which induce apoptosis. The method employs the measurement of induced cysteine aspartic acid protease activity in a cell line that expresses the Fas antigen and has been engineered to overexpress the cell survival polypeptide Bcl-2 and which has been treated with anti-Fas antibody. In a normal unengineered cell line, treatment with the anti-Fas antibody causes rapid apoptosis. However, in the engineered cell line apoptotic progression is blocked due to the continued presence of Bcl-2. Compounds that induce apoptosis are identified by incubating them with the engineered cell line that has also been incubated with anti-Fas antibody and then measuring apoptosis using the method described above. Due to the overexpression of Bcl-2, this method for identifying inducers of apoptosis will identify compounds that either inhibit the function of Bcl-2, or that stimulate the cell death pathway downstream of the Bcl-2 blockade. Moreover, since the engineered cells are treated with the pro-apoptotic anti-Fas antibody, these cells are primed for programmed cell death so that there is little or no lag time once the cells are treated with a positive apoptotic inducer. This priming of the cells provides additional sensitivity and speed for identifying apoptotic inducers. The priming for cell death can be provided by other pro-apoptotic stimuli including but not limited to, staurosporine, TNF and TNF plus cycloheximide.

Detailed Description Text (5):
In another embodiment, the invention is directed to a method of identifying inhibitors of apoptosis. This method also utilizes the single-step method described above, however, the indicator cell line does not require the overexpression of a cell survival polypeptide. Instead, the cells are first incubated with a compound to be tested for inhibitory activity and then treated with an direct stimulus of apoptosis such as anti-Fas antibody. In the absence inhibitory compounds, cells subsequently treated with anti-Fas antibody undergo apoptosis and exhibit a rapid induction of aspartate specific cysteine protease activity. In contrast, positive compounds inhibit the emergence of protease activity compared to control samples.

Detailed Description Text (10):
As used herein, the term "direct stimulus" when used in reference to the cell death pathway is intended to mean an agent that increases the specific apoptotic activity of a cell. Specific examples of direct stimuli include, for example, Fas ligand, anti-fas antibody, staurosporine, ultraviolet (UV) and gamma irradiation. Other direct stimuli exist and are known by those skilled in the art. Thus, a direct stimulus of apoptosis is an agent which increases the molecular activity of the above-described families of molecules which enhance or participate in apoptosis.

Detailed Description Text (27):
Therefore, the invention provides the measurement of specific apoptotic activity in a cell population with as few as 10,000 and as many as 1.times.10.sup.6 cells. Usually

the cell population is greater than about 50,000 to allow for relatively simple detection and preferably the cell population is about 100,000. Of course, the cell population can be further decreased compared to those sizes given above, and the method can be compensated for by allowing a slightly longer incubation time with the apoptotic stimulus, detection reagent and/or both. Thus, there are a variety of alternatives available to those skilled in the art which can be employed to practice the invention.

Detailed Description Text (41):
The holding point which prevents the cell from proceeding into programmed cell death is the overexpression of a cell survival polypeptide. Cell survival polypeptides are characterized in that they exhibit the ability to prevent apoptosis when expressed or activated in a cell induced to undergo apoptosis. For example, in the absence of a functioning cell survival polypeptide, a cell treated with an apoptotic inducer will initiate the programmed cell death pathway and eventually die by apoptosis. However, in the presence of a cell survival polypeptide, treatment with an apoptotic inducer can initiate the programmed cell death pathway but the cell will survive due to inhibition of one or more events along the pathway. Depending upon the point at which the cell survival polypeptide functions, the programmed cell death pathway can be inhibited early or relatively late within the execution of the cascade of events leading to ultimate cell death. Cell survival polypeptides and their encoding nucleic acids are well known in the art and include, for example, the Bcl-2 family of related proteins Bcl-2, Bcl-xL, Mcl-1, E1B-19K as well as inhibitors of the caspases such as p35, crmA and the dominant-negative forms of the caspases. These forms include, for example, caspase's with an inactivating mutation of the active site cysteine.

Detailed Description Text (43):
A level of expression of a cell survival polypeptide which is sufficient to prevent the induction of apoptosis is known to those skilled in the art and can also be routinely determined by those skilled in the art. Expression vectors and systems are known and commercially available which provide for recombinant polypeptide expression. It is a routine matter for one skilled in the art to choose a vector or system which will provide sufficient levels of expression in a particular host cell. Alternatively, the expression level sufficient to prevent the induction of apoptosis can be routinely determined by expressing the cell survival polypeptide and then measuring whether the cell survives after treatment with an apoptotic stimulus.

Detailed Description Text (45):
The block from apoptosis due to overexpression of a cell survival polypeptide and the treatment of the cells with a direct stimulus of apoptosis provide antagonistic influences to the cell. In this way, the cells are then essentially poised for programmed cell death. A direct stimulus for apoptosis can be a variety of different insults to the cell including, molecular, environmental and physical stimuli. As defined previously, such stimuli are known to those skilled in the art and can be characterized by activating a molecule within the apoptotic pathway. Examples of direct stimuli of apoptosis include inducers such as Fas ligand, anti-Fas antibody, Staurosporine, ultraviolet and gamma irradiation. Thus, treatment of a cell over expressing a cell survival polypeptide with a direct stimulus of apoptosis will prime the cell for apoptosis since both positive and negative signals are providing balancing effects. One advantage of this priming is that all cell death components are available for apoptosis once a signal is received that overrides the block of the cell survival polypeptide. This advantage allows for the rapid induction of apoptosis which can be beneficial when used to screen for compounds that possess apoptosis inducing activity when Bcl-2 or Bcl-xL is the cell survival polypeptide. Such cells are particularly useful in screening for inhibitors of Bcl-2 or Bcl-xL, respectively.

Detailed Description Text (46):
In addition to treating the cells with a direct stimulus of apoptosis, the cells are also treated with one or more compounds which are to be tested for apoptosis inducing activity. The compounds can be, for example, small molecules, peptides, polypeptides, proteins or other macromolecules. Essentially, the type of compound which is to be tested is unimportant, only that the user desires to test whether the compound has apoptotic inducing activity. Therefore, the assay is applicable for a variety of different settings, including clinical, diagnostic and drug discovery.

Detailed Description Text (58):
Studies were initially carried out comparing the single-well method described above (where the entire experiment is carried out in the same well), with a known method where cells are spun down and washed after anti-Fas antibody treatment and before addition of lysis buffer. This comparison is presented in FIG. 1A. The results show

that both methods yield comparable caspase activity as demonstrated by the CPP32-like enzymatic cleavage of the DEVD-AMC (SEQ ID NO:1) substrate in neo-transformed cell lines. In contrast, cell lines transfected with Bcl-2 are protected from Fas induced death and show little increase in cleavage (FIG. 1B). These results demonstrate that the single-well method can accurately measure both the induction and inhibition of caspase activity at a sensitivity comparable to previously used methods.

CLAIMS:

4. The method of claim 1, wherein said compound exhibits caspase inhibitory activity.

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L3: Entry 17 of 25 File: USPT Jun 26, 2001

US-PAT-NO: 6251614
DOCUMENT-IDENTIFIER: US 6251614 B1

TITLE: Rapid methods for identifying modifiers of cellular apoptosis activity

DATE-ISSUED: June 26, 2001

INVENTOR-INFORMATION:

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APPL-NO: 09/ 309003 [PALM]
DATE FILED: May 10, 1999

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATION This application is a divisional of pending U.S. patent application Ser. No. 08/869,553, filed Jun. 5, 1997.

INT-CL: [07] C12 Q 1/00, C12 Q 1/02, C12 Q 1/37

US-CL-ISSUED: 435/7.2; 435/4, 435/7.21, 435/23, 435/24, 435/29, 435/32, 435/375, 435/377, 436/63
US-CL-CURRENT: 435/7.2; 435/23, 435/24, 435/29, 435/32, 435/375, 435/377, 435/4, 435/7.21, 436/63

FIELD-OF-SEARCH: 435/4, 435/7.21, 435/23, 435/24, 435/29, 435/32, 435/375, 435/377, 435/7.2, 436/63

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5620888	April 1997	Tomei	435/325
☐	5624808	April 1997	Thompson et al.	435/7.24
☐	5637465	June 1997	Trauth	435/7.1
☐	5783186	July 1998	Arakawa et al.	424/143.1
☐	5834216	November 1998	Ruizman et al.	435/7.21

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 95/27903	October 1995	WO	
WO 96/26280	August 1996	WO	
WO 96/35124	November 1996	WO	
WO 97/06182	February 1997	WO	
WO 97/08174	March 1997	WO	
WO 97/09617	March 1997	WO	
WO 97/10349	March 1997	WO	
WO 97/18313	May 1997	WO	
WO 98/13690	April 1998	WO	

OTHER PUBLICATIONS

Armstrong et al., "Fas-induced Activation of the Cell Death-related Protease CPP32 Is Inhibited by Bcl-2 and by ICE Family Protease Inhibitors," The Journal of Biological Chemistry 271(25):16850-16855, 1996.
Enari et al., "Apoptosis by a cytosolic extract from Fas-activated cells," The EMBO Journal 14(21): 5201-5208, 1995.
Hockenbery et al., "Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death," Nature 348: 334-336, 1990.
Los et al., "Requirement of an ICE/CED-3 protease for Fas/APO-1-mediated apoptosis," Nature 375:81-83, 1995.

ART-UNIT: 164

PRIMARY-EXAMINER: Saunders; David

ABSTRACT:

The invention provides a single-well, microscale method of determining the specific apoptotic activity of a cell. The method consists of contacting a cell population of about 1.times.10.sup.5 cells for a time period of between about 30 minutes and 4 hours with a sufficient volume of medium containing an apoptotic specific diagnostic reagent and a diagnostic accessory reagent so as to cover the cell population, and determining the activity of the apoptotic specific diagnostic reagent. The invention also provides a method of identifying a compound which induces apoptosis. The invention further provides a rapid method of identifying a compound which inhibits apoptosis.

6 Claims, 5 Drawing figures

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L3: Entry 7 of 25 File: USPT May 21, 2002

DOCUMENT-IDENTIFIER: US 6391612 B1
TITLE: Vectors, cells, and methods for the production of deleterious adenoviral, herpes viral and adeno-associated viral vectors

Detailed Description Text (8):
The blocking gene can be any suitable gene and can be derived from a viral or cellular source. If present on the viral eukaryotic gene transfer vector or another viral vector, preferably, the blocking gene is under the control of a heterologous promoter or is placed in a new trancriptional control unit within the virus. If present on a plasmid or in the genome of the eukaryotic host-production cell, the blocking gene can comprise a native promoter or a heterologous promoter a long as the promoter effects expression of the blocking gene coding sequence. In any event, the blocking gene desirably substantially blocks the deleterious effects on the host-production cell. Examples of blocking genes include, but are not limited to, genes that encode crmA, a caspase inhibitor such as baculoviral p35 or an IAP gene product, a FLIP gene product, and adenoviral 14.7 K protein. For example, with an adenoviral vector, the DNA encoding the 14.7 K protein is preferably moved from its native location in the E3 region to the E1 or E4 region of the adenoviral genome. It is also useful to link operably the DNA encoding the, 14.7 K protein to a more powerful or more regulatable promoter than the native E3 promoter. In this regard, any suitable promoter, e.g., the cytomegalovirus (CMV) immediate early promoter or a host-production cell restricted promoter, can be used to drive the expression of the 14.7 K protein.

Detailed Description Text (21):
Blocking genes that are useful in the context of the present invention are those that encode and express a gene product that directs the expression of a protein that inhibits a caspase. Caspase inhibitors, which are well-known in the art, are reviewed by Nicholson et al., Trends Biol. Sci. (TIBS), 22, 299-306 (1997). The blocking gene product can directly inhibit the caspase or can function by acting on other points in the caspase pathway. The caspase inhibitor preferably inhibits the caspase protease by binding directly to the caspase. More preferably, the caspase inhibitor binds to a region of the caspase that comprises the protease cleavage site. Examples of caspase inhibitors useful in the context of the present invention include crmA, adenoviral 14.7 K protein, baculoviral p35 protein, IAP gene products and FLIP gene products. Adenoviral 14.7 K protein, baculoviral p35 protein and especially crmA are particularly useful in the context of the present invention.

Detailed Description Text (25):
The present inventive method and cells can be augmented by the use or application of peptide mimetic inhibitors of caspases. Peptide mimetics suitable for use in the context of the present invention include (1) acetyl-aspartyl-glutamyl-valinyl-aspartic aldehyde, (2) carbobenzoxy-L-aspartyl-.alpha.-(2,6 dichlorobenzoyl) methane (Z-Asp-CH.sub.2 -DCB), and (3) carbobenzoxy-valinyl-alaninyl-aspartyl methoxyfluromethane (Z-VAD-FMK). Although these peptide mimetics are generally very expensive, especially when preparing commercial quantities of a viral eukaryotic gene transfer vector, addition of these mimetics to the medium of a host-production cell can decrease the cytotoxicity, cytostasis, or apoptosis of a host-production cell.

Detailed Description Text (51):
HEK-293 cells and A549 cells were transiently transfected with three plasmids: one that expresses Green Fluorescent Protein (GFP); one that expresses FasL, FADD or FLICE, and one that expresses either 14.7 K protein or crmA protein. The expression of GFP in the cells makes it easy to assay the cells for apoptosis, which was measured about 12 hrs after transfection. FasL, of course, induces apoptosis through the Fas ligand receptor. Similarly, FADD and FLICE are well-known in the art to stimulate apoptosis.

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L5: Entry 8 of 10 File: USPT Mar 14, 2000

DOCUMENT-IDENTIFIER: US 6037461 A
TITLE: FADD-like anti-apoptotic molecules, methods of using the same, and compositions for and methods of making the same

DATE FILED (1):
19970520

Detailed Description Text (6):
The discovery of the two FLAMEs provides the means to design and discover specific inhibitors, activators and substrates of these anti-apoptotic molecules. According to the present invention, FLAMEs may be used to screen compounds for inhibitors, activators or substrates. Inhibitors are useful as apoptotic agents. Activators are useful as anti-apoptotic agents. FLAME-1 and FLAME-2 proteins are useful as reagents in assays to identify inhibitors and activators as well as in binding assays such as FLAME-1 binding assays with FADD, Mch4, Mch5 and FLAME-2 and FLAME-2 binding assays with Mch4, Mch5 and FLAME-1. FLAME-1 may also be useful as a substrate for caspase in assays to identify caspase inhibitors. Kits are provided for screening compounds for FLAMEs inhibitors. Kits are provided for screening compounds for FLAMEs activators. Kits are provided for screening compounds for FLAME binding assays. The nucleotide sequences that encode the FLAMEs are disclosed herein and allow for the production of pure protein, the design of probes which specifically hybridize to nucleic acid molecules that encode the FLAMEs and antisense compounds to inhibit transcription of FLAMEs. Anti-FLAME-1 and anti-FLAME-2 antibodies are provided. Anti-FLAME-1 antibodies may be inhibitors of FLAME-1 and may be used in methods of isolating pure FLAME-1 and methods of inhibiting FLAME-1 activity. Anti-FLAME-2 antibodies may be inhibitors of FLAME-2 and may be used in methods of isolating pure FLAME-2 and methods of inhibiting FLAME-1 activity.

Detailed Description Text (79):
Transfection studies showed that FLAME-1 may also be a caspase substrate in vivo. Expression of a T7-epitope tagged FLAME-1 (T7-FLAME-1) in 293 cells produced both full length and cleaved (p39) FLAME-1 (See FIGS. 3B and 3F). This cleavage was not observed with the D341A mutant FLAME-1 (T7-FLAME-1-D341A, see FIGS. 3C and 3F). Furthermore, stimulation of FLAME-1-transfected MCF7-FAS cells with anti-Fas antibody increased the amount of cleavage products. Thus, FLAME-1 appears to be a caspase target in apoptotic cells.

Print Request Result(s)

Printer Name: cm1_9e12_gbefptr
Printer Location: cm1__9e12

- US006251614: Ok
- US006403765: Ok
- US006228603: Ok

OK | Back to List | Logout

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End of Result Set

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L5: Entry 10 of 10 File: USPT Jan 12, 1999

DOCUMENT-IDENTIFIER: US 5858778 A
TITLE: SF caspase-1 and compositions for making and methods of using the same

DATE FILED (1):
19961227

Abstract Text (1):
A substantially pure protein, Caspase-1, is disclosed. An isolated nucleic acid molecule that comprises a nucleic acid sequence that encodes Caspase-1, is disclosed. An isolated nucleic acid molecule consisting of a nucleic acid sequence that encodes Caspase-1, or a fragment thereof having at least 10 nucleotides is disclosed. Recombinant expression vector comprising a nucleic acid sequence that encodes Caspase-1 and host cells comprising the recombinant expression vector are disclosed. Oligonucleotide molecule comprising a nucleotide sequence complimentary to a nucleic acid sequence that encodes Caspase-1 of at least 5 nucleotides are disclosed. Antibodies that binds to an epitope on Caspase-1 are disclosed. Methods of identifying modulators and substrates of Caspase-1 are disclosed.

Detailed Description Text (30):
According to one aspect of the invention, compounds may be screened to identify compounds that inhibit or enhance Caspase-1 activity. Substrates of Caspase-1 include baculovirus protein p35 and the Sf immunophillin FKBP46. Assays may be performed combining Caspase-1 with a substrate in the presence or absence of a test compound. The level of Caspase-1 activity in the presence of the test compound is compared to the level in the absence of the test compound. If Caspase-1 activity is increased by the presence of the test compound, the test compound is an enhancer. Caspase-1 activity is decreased by the presence of the test compound, the test compound is an inhibitor. In some embodiments of the invention, the preferred concentration of test compound is between 1 .mu.M and 500.mu.M. A preferred concentration is 10 .mu.M to 100 .mu.M. In some preferred embodiments, it is desirable to use a series of dilutions of test compounds.

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L5: Entry 9 of 10 File: USPT Jan 19, 1999

DOCUMENT-IDENTIFIER: US 5861498 A
TITLE: Nucleotides encoding immunophilin FKBP46 and fragments thereof

DATE FILED (1):
19961031

Detailed Description Text (35):
It has been discovered that FKBP46 is a substrate for Caspase-1, a novel Sf9 insect cell protease that is activated in apoptosis. The processing of FKBP46 may be involved in cell death.